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ECOLOGY OF ARBOVIRUSES IN A MARYLAND FRESHWATER SWAMP

II. BLOOD FEEDING PATTERNS OF POTENTIAL MOSQUITO VECTORS

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LeDuc, J. W., W. Suyemoto, B. F. Eldridge and E. S. Saugstad (Walter Reed Army Institute of Research, Wash., D.C. 20012). Ecology of arboviruses in a Maryland freshwater swamp. II. Blood feeding patterns of potential mosquito vectors. *Am J Epidemiol* 96: 123–128, 1972.— Blood engorged mosquito specimens from the Pocomoke Cypress Swamp, Maryland, were collected from May through November, 1969. Five mosquito species (*Aedes atlanticus, Aedes canadensis, Culex salinarius, Culiseta melanura* and *Psorophora ferox*) were examined by capillary type precipitin tests of engorged material and by comparisons of human biting collections and collections of mosquitoes attracted to caged animals. *A. atlanticus* and *P. ferox* had similar feeding patterns, both most frequently feeding on sylvatic mammals, while *C. salinarius* appeared to feed mostly on domestic mammals adjacent to the swamp. *A. canadensis* was an omnivorus feeder, while *C. melanura* fed almost exclusively on birds. The potential of these mosquitoes as vectors of arboviruses known to be present in the swamp is discussed.

arbovirus; disease vectors; ecology; encephalitis, equine; mosquitoes; viruses

Introduction

The endemic nature of arboviruses in the Pocomoke Cypress Swamp has been established. Eastern and western equine encephalitis viruses (EEE, WEE) have been isolated from pooled *Culiseta melanura* (Coquillett) (1, 2) and other mosquito species have yielded undetermined arbovirus agents (Suyemoto, W., unpublished data). As part of an effort to determine the introduction, transmission and maintenance of arboviruses in the swamp, feeding patterns of potential mosquito vectors were analyzed. It was hoped that mosquito blood meal analyses might provide clues to those mosquito species which are significant in the cycle of virus transmission in nature and provide insight into virus over-wintering mechanisms and maintenance of the virus within the sylvatic habitat. Finally, the potential of a mosquito species, from a standpoint of its feeding behavior, to transmit viruses to man or domestic animals was considered.

Materials and methods

Female mosquitoes were collected from May through November, 1969, employing a variety of collection methods. These methods included CDC miniature light traps

Abbreviations: CEV, California encephalitis virus; EEE, eastern equine encephalitis; WEE, western equine encephalitis.

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with and without dry ice, and D-Vac (D-Vac Co., Riverside, Calif.) vacuum sweeps around caged and sentinel animals. Resting boxes, Malaise traps, net sweeps, and human bait collections were also used.

The collection techniques were categorized as to bias based on the collection method employed. Those methods considered biased were: 1) vacuum sweeps around caged animals made regularly throughout the year and infrequent collections around domestic animals in areas on the periphery of the study area, and 2) human bait collections made twice weekly on a rotational basis among the major habitat study areas for a one hour period near sunset. These collections included engorged and unengorged females, and indicate only a degree of attraction of the mosquitoes collected for the host sampled. Methods which are considered less biased from the point of view of feeding preferences, were collections made weekly at permanent light trap sites, from resting boxes, and from vacuum sweeps in areas away from any caged animals. These collections consisted only of blood-engorged females.

Blood engorged specimens were transported to a base laboratory and killed by chilling in a −20 C freezer. They were then sorted over wet ice and stored in a freezer at −60 C. After species identification, abdomens were removed and placed in gelatin capsules. These were then refrozen and the remainder of the mosquito pooled with others of that collection for virus isolation attempts.

The trituration and testing of engorged mosquitoes followed the techniques described by Tempelis and Lofy (3). Only antisera with a minimum homologous titer of 1:10,000 and no crossing with heterologous antigens at 1:1,000 were used. All screening antisera were prepared in rabbits and specific antisera in roosters. Precipitin tests were conducted in capillary tubes with readings recorded at one and two hours and then overnight. Controls were set up daily for all antisera used. Where crossing occurred with anti-deer, anti-goat, and anti-bovine antisera with heterologous antigens, the cross reactions were virtually eliminated by serial twofold dilutions of the antisera up to 1:16 using phosphate buffered saline, the same diluent as used to grind mosquito abdomens. Where crossing could not be eliminated at this dilution, test results were recorded as deer-bovine, deer-goat or deer-goat-bovine. All multiple feeding results were retested.

Results

Definite feeding patterns are evident in the tabulated data of biased and unbiased collections. *Aedes canadensis* was found to be very catholic in its feeding habits. This species fed readily on mammals and reptiles, and to some extent on birds (table 1, figure 1), and was found to be frequently attracted to humans (table 2). Especially noteworthy was the extremely high rate of deer feeding, comprising about 47 per cent of the total feeds, or 61 per cent of the unbiased mammal positives (table 1).

While *A. atlanticus* apparently did not constitute a large percentage of the mosquito population (1), we did find it to be frequently attracted to sentinel rabbits and other small mammals (table 2). This pattern is evident in both the biased and unbiased collections. Although this mosquito fed on deer, it was not collected from pigs, goats or cows, all of which were found adjacent to the swamp (table 2). It was frequently attracted to humans. Reptiles appeared to play a slight role as hosts of this species whereas birds did not (figure 1).

Although *Culex salinarius* was common in the study area (1), this species was infrequently encountered engorged by our collection methods. When collected, it was most often found near domestic animals on the periphery of the swamp (table 2). Neither birds nor reptiles appeared to be significant hosts of this mosquito in our study area (figure 1).

The feeding patterns of *A. atlanticus* and *Psorophora ferox* were similar. Both species

were attracted to small mammals and humans and to a lesser extent to birds (figure 1). Both species fed with equal frequency on sentinel rabbits, but *P. ferox* fed more frequently on caged rats. Although attracted to them, it did not feed on reptiles (table 1, 2). Our less biased samples of engorged *P. ferox* specimens were too small to ascertain with certainty its natural feeding habits (table 1).

Culiseta melanura showed the least variation in its host selection of any of the mosquitoes studied. Over 90 per cent of the *C. melanura* tested were found to contain avian blood, the majority of these being from passerine birds (figure 1). Occasional quail fed specimens were found, but no chicken positives were, even though chickens were present on a farm near the swamp (table 1). All mammal positives were found in late summer or fall, after the main peak of abundance of the *C. melanura* population was past. No reptile or human positives were found (table 1).

TABLE 1

Summary of mosquito feeding patterns as sampled by light traps, resting boxes and net sweeps. Pocomoke Cypress Swamp, Maryland, 1969

Host	*Aedes canadensis*	*Aedes atlanticus*	*Culex salinarius*	*Culiseta melanura*	*Psorophora ferox*
Human	4	0	0	0	0
Pig	7	0	0	0	0
Deer	315	15	11	3	2
Goat	11	0	9	0	0
Bovine	13	1	2	0	0
Deer/bovine	9	0	0	0	0
Deer/goat	4	0	0	0	0
Deer/goat/bovine	1	0	0	0	0
Dog	5	0	1	0	0
Raccoon	12	1	0	1	2
Rat	11	0	1	0	0
Squirrel	2	0	0	0	0
Rat/squirrel	1	0	0	0	0
Rabbit	55	6	3	1	0
Opossum	7	0	1	0	0
Undetermined mammal	58	2	2	0	1
Passeriformes	22	0	0	272	0
Columbiformes	2	0	0	0	0
Galliformes	1	0	0	10	0
Other orders*	1	0	0	25	0
Undetermined bird	15	0	2	23	1
Reptile	113	1	0	0	0
Total	669	26	32	335	6

* Included Anseriformes, Charadiformes and Ciconiiformes.

DISCUSSION

The omnivorous feeding habits and abundance of *A. canadensis* indicate its potential as a vector of arboviruses. The degree of feeding by *A. canadensis* on deer and the reported deer involvement with California encephalitis virus (CEV), WEE and Cache Valley agents by Whitney et al. (4) again suggest ideal vector potential from the standpoint of range of host feeding. In 1969 the population of *A. canadensis* was biphasic, consisting of a peak in late spring-early summer and another in late summer (1). Throughout most of these population fluctuations the per cent of mammal feeding was about 77 per cent, reptiles 17 per cent and birds about 6 per cent. However, in late spring this pattern was altered by an increase in reptilian feeding to abut 22 per cent, while mammalian feeding dropped to about 68 per cent. We find this observation to be consistent with previously reported findings. Hayes (5) noted feeding on turtles in late May, and Nolan et al. (6) in early June. In a more complete study, Crans and Rocke (7) found *A. canadensis* the only species collected from turtles early in the season. We believe that the increased feeding by *A. canadensis* on reptiles, especially turtles, is dependent on host availability and that the apparent increase in reptilian feeding reflects the oviposition behavior of the turtles. In our study area, turtles leave

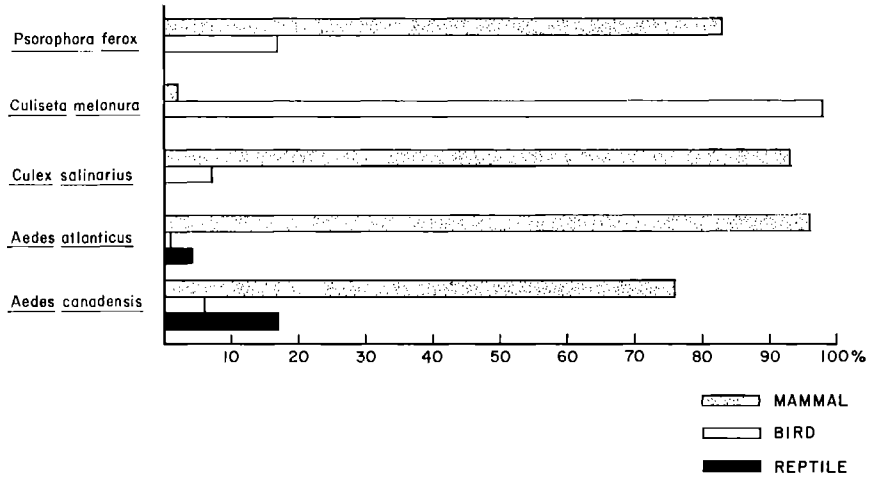

FIGURE 1. Blood-feeding rates of five species of mosquito on three classes of vertebrate hosts as sampled by light traps, resting boxes and net sweeps, Pocomoke Cypress Swamp, Maryland, 1969.

TABLE 2

*Collection frequency of five species of mosquitoes using various animals as bait, Pocomoke Cypress Swamp, Maryland, 1969**

Bait	No. of collections	No. of collections containing				
		Aedes canadensis	*Aedes atlanticus*	*Culex salinarius*	*Culiseta melanura*	*Psorophora ferox*
Human	55	45	24	19	7	15
Pig	1	1	0	1	0	1
Cow & goat	2	2	0	2	2	0
Raccoon	17	14	10	6	7	9
Rat	11	2	3	2	4	11
Rabbit	10	7	9	4	4	9
Opossum	2	1	1	0	0	0
Quail	13	7	8	2	7	6
Turtle	12	9	10	1	5	4
Snake	14	10	6	5	2	4

* Human bait collections include landing and biting females; collections from other bait animals include all females flying in the immediate vicinity of bait.

the swamp habitat in spring and travel to higher ground where the females deposit their eggs. This movement takes them from the protection of the swamp and increases their exposure to mosquito feeding. Should a poikilothermic virus reservoir exist in our area similar to that found by Gebhardt et al. (8), this feeding by *A. canadensis* could be a vehicle by which virus is reintroduced into a warm blooded host cycle.

Though representing a small portion of the mosquito population, the feeding activity of *A. atlanticus* and *P. ferox* becomes important considering their intensive feeding on rabbits. Hoff et al. (9) and others have established substantial involvement of rabbits with CEV. This suggests that these two mosquito species could be significantly involved in the natural transmission of this arbovirus. Feeding on humans by these species implies a vector potential for CEV to man as well. Recent studies in Florida (10) support this contention.

Culex salinarius, on the basis of its feeding patterns, is of potential medical importance. Females of this species, although they fed predominantly on domestic livestock in areas surrounding the swamp, also fed on birds and mammals within the swamp. Occasional isolations of EEE indicate that they do feed on arbovirus hosts (11). The scarcity of virus isolations from this species (none in 1969), in the face of high adult abundance, points to an effective ecological barrier minimizing this species' importance as a vector. Low susceptibility

to infection is probably a contributing factor (12).

Although *C. melanura* is probably responsible for disseminating EEE and WEE viruses throughout the avian population, our findings, showing minimal non-avian feeding, suggest that this species has a low potential for the transmission of these viruses into the mammalian and reptilian vertebrates. It is possible, however, that this feeding pattern may vary under conditions other than those examined. A temporal analysis of *C. melanura* feeding reveals an interesting observation. Although few in number, all mammal feedings were found to occur during the latter part of the year, after the mosquito population peak had subsided. In an intensive study of *C. melanura,* Joseph and Bickley (13) also found the per cent of mammal positives was generally higher from September through November, as the mosquito population was subsiding. The number of birds found in the swamp during this period increases due to fall migration, suggesting that bird availability alone is not the reason for this apparent shift. However, it is possible that *C. melanura* feeds most readily on a particular summer resident. The departure of the summer residents in fall could account for increased feeding on secondary mammalian hosts.

Through preferential feeding on a certain class of vertebrates, each mosquito species is exposed primarily to that group of arboviruses utilizing a particular class of vertebrate hosts as its reservoir or amplifying mechanism. Thus, *C. melanura,* by feeding primarily on avian hosts, is most likely to contract EEE or WEE, both of which are viruses associated with birds. This type of preferential feeding can be seen in figure 1, in which group feeding preferences are graphically compared.

California encephalitis virus group agents have most often been associated with rabbits as their vertebrate reservoir or amplifying mechanism. Comparing the attractiveness to mosquitoes of rabbits against other small mammals demonstrates a preference by certain mosquito species for this host, implying increased exposure to this group of arboviruses. *Aedes atlanticus, A. canadensis,* and *Psorophora ferox* stand out as species attracted to rabbits (table 2).

The relative attractiveness of humans to the five species studied is implied by the frequency of their appearance in human biting collections (table 2). These frequency data exaggerate the attraction of *Culiseta melanura,* however, because although females of this species occurred in over 10 per cent of the human biting collections, their numbers were very small in comparison with the other species, and in no case were they ever observed actually biting.

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